Summary. The adaptation of equine abortion virus to HeLa cells through 35 serial passages is reported. Characteristic intranuclear inclusions occur in cultures selected at random. The agent fixed complement in significant dilutions and remained pathogenic for hamsters.

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Influence of Stress on Distribution of Endotoxin in RES Determined by Fluorescein Antibody Technic.* (23271)

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The site and mode of primary action of endotoxin of Gram negative bacteria in animal tissue is unknown. In an attempt to elucidate this problem, results of parenteral injection of toxin have been studied under a variety of conditions. Among the many experiments, which have been performed, are studies of the effect of toxin after a single injection in normal animals and in animals treated with cortisone, thorotrast, x-irradiation and after two spaced injections(2,8,13,14). Since cortisone and thorotrast can substitute for the preparatory dose of toxin in the generalized Shwartzman reaction, it was considered of interest to determine how animals dispose of toxin under these conditions. Therefore, distribution and retention of the endotoxin of *Salmonella typhosa* after intravenous injection in normal animals and in animals stressed by the aforementioned agents was followed by the Coons' fluorescein technic.†

Materials and methods. The direct fluorescein tagging of gamma globulin according to the method of Coons and Kaplan(5) was employed. Normal and typhoid immune gamma$_2$ globulin fractions were purified by the method of Nichol and Deutsch(11). The endotoxin of *Salmonella typhosa,* strain Edward, was purified by the method of Webster, *et al.* (15). All experiments were performed in American Dutch rabbits weighing approximately 1 kg. The deposition of toxin was studied in 15 normal rabbits after a single intravenous injection of 2 mg of toxin. The animals died or were killed between 20 minutes and 47 hours after injection. In the cortisone study, 15 rabbits were injected intramuscularly daily for 3-4 days with 25 mg of cortisone acetate.‡ After third injection of cortisone, 2 mg of toxin was injected intravenously. The animals died or were killed between 30 minutes and 46 hours after toxin injection. Fourteen animals were injected intravenously with 3 ml of thorotrast§/kg body weight. Six hours later, 2 mg of endotoxin was injected intravenously. The animals died or were killed between 3 hours and approximately 15 hours after toxin injection. Fourteen animals were subjected to 400 roentgen units, total body irradiation. Twenty-four hours later, 2 mg of toxin was injected intravenously. The animals died or were killed

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† The light source was an Osram mercury arc in a Reichert housing equipped with Schott BG 12 filter, 3-3½ mm thick, and a Wratten 2A and 15G filter. We are indebted to Dr. George Price for his assistance in selection of the optical system used.

‡ Cortisone acetate, aqueous suspension, Upjohn Co., Kalamazoo, Mich.

§ Thorotrast, 24-26% thorium dioxide by volume, Testagar and Co., Detroit, Mich.

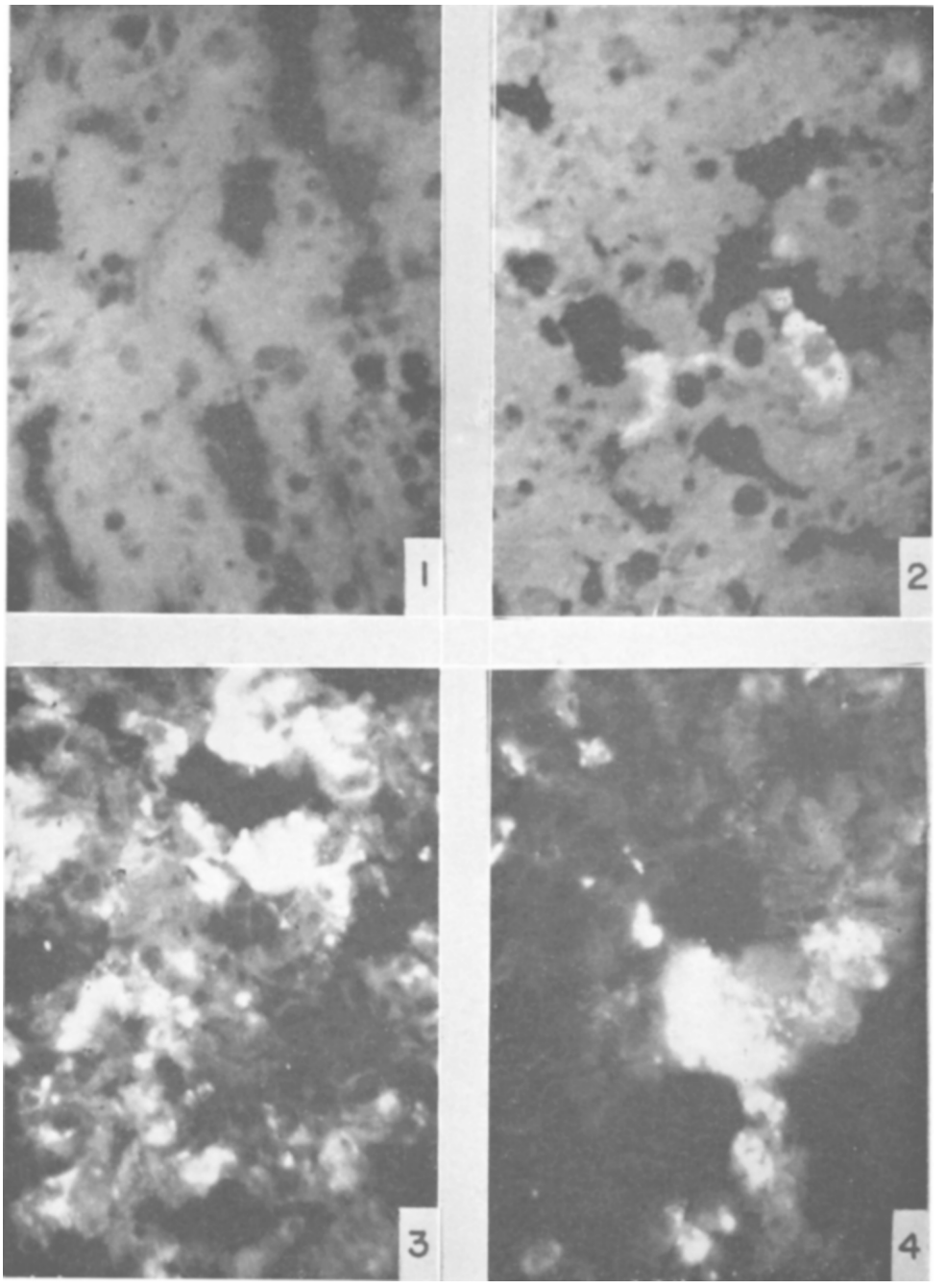

PLATE I. Photomicrographs demonstrating appearance of control and positive tissue sections. Bright areas indicate site of toxin-fluorescein tagged antibody complexes.

1. Duplicate control section of #2 showing inhibition of specific staining by first overlaying section with untagged immune sera and then staining with fluorescein tagged immune gamma globulin. 2. Section of liver stained with fluorescein tagged immune gamma globulin, 1+ reaction. 3. Section of spleen stained with fluorescein tagged immune gamma globulin, 3+ reaction. 4. Section of lung stained with fluorescein tagged immune gamma globulin, 3+ reaction.

between 30 minutes and 54 hours after injection. A series of animals were given an intravenous injection of 250 μg of toxin. Twenty-four hours later the 11 surviving animals were injected intravenously with 2 mg of toxin. The animals died or were killed between 30 minutes and 21 hours after second injection. Blocks of tissue from the liver, spleen, adrenal, lung, kidney and heart were frozen at −70°C and stored at −20°C. Frozen sections, measuring 2-4 μ in thickness, were subsequently cut and stained by the Coons' technic. Three types of controls were employed. Duplicate positive sections were negative when stained with conjugated normal gamma globulin. Specific positive staining was inhibited when the sections were first treated with specific immune, untagged serum. Sections of tissues of uninjected animals and animals which were pretreated with cortisone, thorotrast and x-irradiation, but which had received no toxin, were negative when stained with conjugated immune gamma globulin. The sections were examined microscopically and arbitrarily graded by the amount of toxin observed per high power field. Although it is not possible to obtain quantitative results with this technic, certain obvious and striking differences were observed between normal and stressed animals.

Results. The toxin, when present in any experiment, was found in Kupffer cells of the liver, in the red pulp of the spleen with only an occasional particle in the white pulp, in cells in and near the alveoli of the lung and within the lumen of vessels, in the glomeruli and connective tissue of the kidney, in the connective tissue and in cells lining the vessels of the heart, and in the lining cells of the adrenal sinusoids. In normal animals, the toxin was removed from the circulating blood quickly by the RES, reached a maximum within a short time and then began to decline. The reduction of toxin in the organs with time was most noticeable in the spleen and lungs, the liver maintaining a rather constant pattern over the period of the experiment. Toxin was either absent or minimal in adrenals, heart and kidneys.

In contrast to this, the level of toxin in cortisone treated animals remained high in the spleen and lungs, as well as in the liver, during the period of the experiment. Initial phagocytosis was not curtailed but degradation and elimination of toxin was obviously reduced. An analogous situation occurred in the irradiated animals. Initial phagocytosis was not affected but there was retention of the toxin by the spleen, lung and liver. In both groups of animals the survival time was increased.

The opposite effect was noted in thorotrast treated animals. The RES, engorged with thorotrast particles which fluoresced orange, was able to remove little or none of the circulating toxin. This was reflected in an increased death rate. An impairment of phagocytosis by the RES was also observed in animals given an initial, smaller injection of toxin. The effect was most noticeable in spleen and liver sections. This curtailment of phagocytosis was again reflected in an enhanced death response. However, whereas in the thorotrast experiment, the effect was due to a blockading of the RES by thorium particles, in this experiment, it did not appear to be a blockading effect, since only minimal amounts of toxin were observed in the RES after the first injection.

With an occasional exception, the heart, adrenals and kidneys of all animals showed only a minimal amount of toxin. Nine animals exhibited bilateral cortical necrosis of the kidney, three each from the groups treated with cortisone, x-irradiation and a preliminary injection of toxin. Gross changes in the kidney were not correlated with large accumulation of toxin in the organ.

Discussion. Studies on distribution in animals of endotoxin labelled with radioactive isotopes have been reported(1,3,12). The findings of this study, employing a technic dependent upon immunologic specificity, are essentially in agreement with these earlier reports.

There is a certain analogy between the ability of cortisone, thorotrast, and an initial injection of toxin to prepare for the Shwartzman reaction and the results in this study. Thorotrast and the first injection of toxin by incapacitating the initial phagocytic ability of the RES, probably keeps the provoking dose

TABLE I. Distribution of Endotoxin with Time in Normal and Stressed Animals.

No. of animals	Treatment	Death	Died/killed	Liver	Spleen	Lung	Heart	Adrenal	Kidney
5	None	20 min.– 5 hr	3/2	1–2+	1–2+	2+	±	±	±
10	"	10 hr –47 hr	4/6	1+	+	+	±	±	±
3	Cortisone	30 min.– 2 hr	2/1	1+	3+	2+	±	+	±
12	"	22 hr –46 hr	1/11	1+	2–3+	1+	±	±	±
8	X-irradiation	30 min.– 5 hr	4/4	1–2+	2+	1+	±	±	±
6	"	8 hr –54 hr	0/6	1+	2+	1+	±	+	±
8	Thorotrast	3 hr – 5 hr	4/4	±	±*	+	±	±	±
6	"	6 hr –15 hr	6/0	+	±*	+	±	±	±
9	2 spaced inj. of toxin	30 min.– 5 hr	9/0	+	+	1+	±	±	±
2		7 hr –21 hr	1/1	1+	1–2+	1+	±	±	±

* Majority were negative.

of toxin in the systemic circulation where it can effect its deleterious action at its primary site for a longer period of time. Cortisone, while it does not affect initial phagocytosis, curtails normal degradation and elimination of toxin thus providing a potential, constant source of reinoculation. With normal cell breakdown, the toxin is released and enters the circulation, free once again to act at its primary site. That the toxin remains antigenically unchanged during its sojourn in the RES is evidenced by the fact that the fluorescein tagged antibody specifically complexes with it during this period. This interruption of normal metabolism of toxin by the RES was found also in irradiated animals. Similar observations regarding digestive curtailment of the RES in cortisone treated and irradiated animals are reported by other investigators(4,6,7,9,10). The reinfections, continuing bacteremia, activation of quiescent infections in cortisone and x-rayed animals could be explained on this basis.

Summary. 1. Deposition of endotoxin of *Salmonella typhosa* was followed in normal and stressed rabbits by the fluorescein tagging technic. 2. Pretreatment with cortisone and x-irradiation did not affect initial phagocytosis of toxin but inhibited degradation and elimination of toxin by the RES. 3. Pretreatment with thorotrast and preliminary injection of toxin caused depression of initial phagocytic functioning of the RES to a subsequent injection of toxin. 4. Our findings in their possible relation to the generalized Shwartzman reaction were discussed.

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